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Supplementary Material

Content:

Table S1: Loci in both the ST239 and USA300 collections identified by GWAS as interacting epistatically with the mutation in *ileS.*

ST239

SNP position	Locus Tag/Gene Name	Putative function
1360889	SAWT20_12700	Putative DNA translocase (FtsK/SpoIIIE family protein)
1474673	SATW20_13760	Haloacid dehalogenase-like hydrolase superfamily protein
1536348	*ebh*	Very large surface anchored protein
1557020	*ebh*	Very large surface anchored protein
1557275	*ebh*	Very large surface anchored protein
1579791	*pbp2*	Penicillin-binding protein 2
1937314	SATW20_17780	Putative exported protein
1941832	SATW20_17820	Conserved hypothetical protein
1975607	SATW20_18180	Lantibiotic biosynthesis protein
2033565	SATW20_18600	ABC transporter ATP-binding protein
2075672	*pcrB*	pcrB family protein
2128192	SATW20_19530	β converting phage protein
2312226	SATW20_21880	ABC transporter ATP-binding protein
2409540	SATW20_22770	putative non-haem iron-containing ferritin
2432219	*fmtB*	LPXTG surface-anchored protein
2450342	Intergenic between SATW20_r160 AND SATW20_23010	16S rRNA AND conserved hypothetical protein
2548323	*modC*	Putative molybdenum transport ATP-binding protein
2578126	SATW20_24400	Putative bifunctional protein
2639747	*lldP2*	Putative L-lactate permease 2
2657438	Intergenic between SATW20_25130 AND *gltT*	Putative exported protein AND putative proton/sodium-glutamate symport protein
2674904	*nasD*	Nitrite reductase large subunit
2759775	SATW20_26050	Putative short chain dehydrogenase
2790429	SATW20_26280	Conserved hypothetical protein
2810368	Intergenic between SATW20_26460 AND SATW20_26470	Putative haloacid dehalogenase-like hydrolase AND ABC transporter ATP-binding protein
2970902	SATW20_27860	Hypothetical protein
3002241	*hisH*	Putative amidotransferase
3002845	*hisB*	Putative imidazoleglycerol-phosphatedehydratase

Table S1 (continued): Loci in both the ST239 and USA300 collections identified by GWAS as interacting epistatically with the mutation in *ileS.*

USA300

SNP position	Locus Tag/Gene Name	Putative function
61025	SAUSA300_0050	Hypothetical protein
182746	*cap5I*	Capsular polysaccharide biosynthesis protein
260357	Intergenic between SAUSA300_0219 AND *pflB*	Putative iron compound A C transporter, iron compound-binding protein AND formate acetyltransferase
270510	SAUSA300_0226	3-hydroxyacyl-CoA dehydrogenase
289635	SAUSA300_0239	PTS system, fructose-specific enzyme II, BC component
292738	*gutB*	Sorbitol dehydrogenase
331125	SAUSA300_0279	Putative membrane protein
351171	SAUSA300_0300	Conserved hypothetical protein
406847	SAUSA300_0355	Acetyl-CoA acetyltransferase
429073	*ahpF*	Alkyl hydroperoxide reductase, subunit F
467549	SAUSA300_0414	Staphylococcal tandem lipoprotein
480640	SAUSA300_0426	Conserved hypothetical protein
577067	*cysE*	Serine acetyltransferase
635299	*vraB*	Acetyl-CoA c-acetyltransferase
742782	Intergenic between SAUSA300_0669 AND SAUSA300_0670	Undecaprenol kinase AND ABC transporter, ATP-binding protein, MsbA family
944770	Intergenic between *argG* AND *pgi*	Argininosuccinate synthase AND glucose-6-phosphate isomerase
1215913	*sun*	Ribosomal RNA small subunit methyltransferase B
1286986	*ftsK*	DNA translocase FtsK
1295497	*cinA*	Competence/damage-inducible protein cinA
1363207	*sbcC*	Exonuclease SbcC
1568945	SAUSA300_1403	phiSLT ORF412-like protein, portal protein
1632033	Intergenic between SAUSA300_1477 AND SAUSA300_1478	Transposase AND lipoprotein
1640549	SAUSA300_1485	Conserved hypothetical protein
1944161	Intergenic between *splA* AND SAUSA300_1759	Serine protease AND hypothetical protein
1961241	SAUSA300_1778	tRNA-asp
2102731	SAUSA300_1934	phi77 ORF020-like protein, phage major tail protein
2275521	SAUSA300_2106	Putative transcriptional repressor
2276564	*mtlA*	PTS system, mannitol specific IIA component

2308707	SAUSA300_2135	Iron compound ABC transporter, permease protein
2322700	SAUSA300_2146	Alcohol dehydrogenase, zinc-containing
2685939	SAUSA300_2486	Putative ATP-dependent Clp proteinase
2700617	SAUSA300_2497	Aminotransferase, class I
2803422	SAUSA300_2583	Putative glycosyl transferase

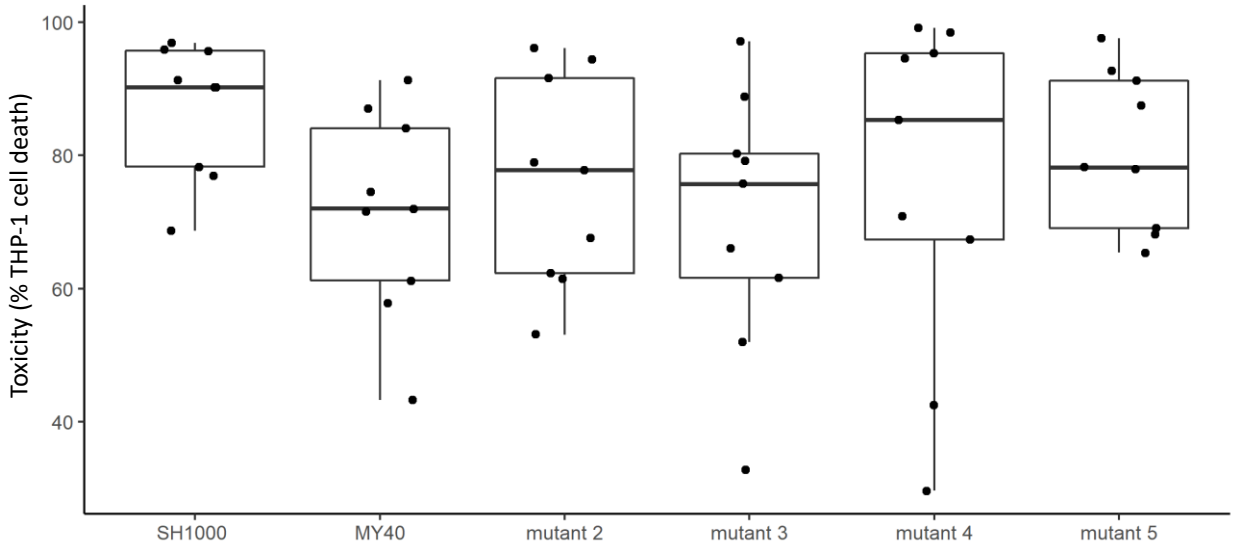

Fig. S1: The mup^{R} mutation reduces the toxicity of the *S. aureus* strain SH1000. The toxicity of five independent mup^{R} isolates, each with the V588F conferring SNP were quantified, in each case their ability to lyse THP-1 cells was reduced upon acquisition of this resistance SNP.

Table S2: DNA sequence results for SH1000 and MY40. The strains were sequenced and mapped to the closest reference strain NCTC8325. Presented here are the SNP differences between SH1000 and MY40 having filtered out all the differences they had in common with respect to the reference strain. The only non-synonymous change was the mupirocin resistance conferring mutation in the *ileS* gene. (CDS: coding sequences; N/A: not applicable; hypo: hypothetical; Syn: synonymous)

SNP position	Feature	Gene name/locus-tag	Nucleotide in SH1000	Nucleotide in MY40	Amino acid change
75276	Intergenic	N/A	T	A	N/A
290135	CDS	SAOUHSC_00270 (hypo. protein)	A	G	Syn
290144	CDS	SAOUHSC_00270 (hypo. protein)	T	A	Syn
290147	CDS	SAOUHSC_00270 (hypo. protein)	T	C	Syn
290149	CDS	SAOUHSC_00270 (hypo. protein)	C	T	Syn
405366	Intergenic	N/A	G	A	N/A
841103	CDS	SAOUHSC_00877 (hypo. protein)	G	T	syn
841139	CDS	SAOUHSC_00877 (hypo. protein)	G	T	Syn
1108891	CDS	Isoleucyl-tRNA synthetase	G	T	V588F
1562913	Intergenic	N/A	T	A	N/A
2134749	CDS	SAOUHSC_A02189 (hypo. protein)	T	C	Syn
2244467	Intergenic	N/A	G	A	N/A
2244495	Intergenic	N/A	A	G	N/A
2661023	CDS	SAOUHSC_02887 (hypo. protein)	C	T	Syn

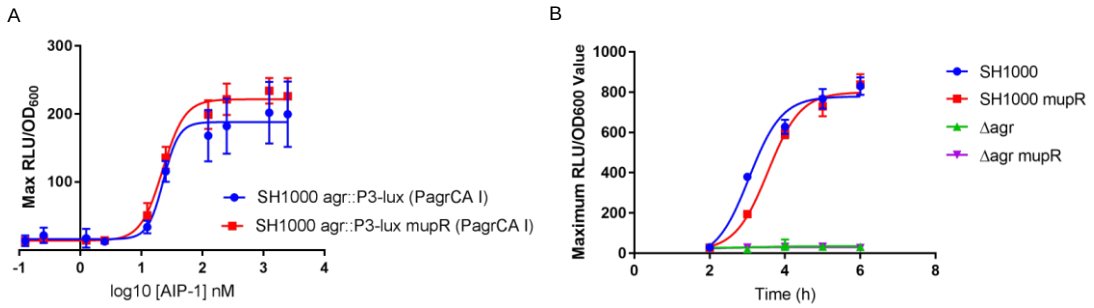

Fig. S2: Agr activity is not affected by the mup^{R} mutation. Effect of mupirocin resistance on the response to exogenous AIP, and on AIP production as a function of growth. **A**: The half maximal effective concentration (EC_{50}) of AIP required to activate the Agr system was quantified for both the mupirocin resistant and sensitive *S. aureus* strains, where no significant effect of mupirocin resistance was observed. **B**: The relative concentration of AIP in the culture supernatant was quantified following growth for 6h for both the mupirocin resistant and sensitive strains. No effect of mupirocin resistance on this aspect of Agr activity was observed under these conditions.

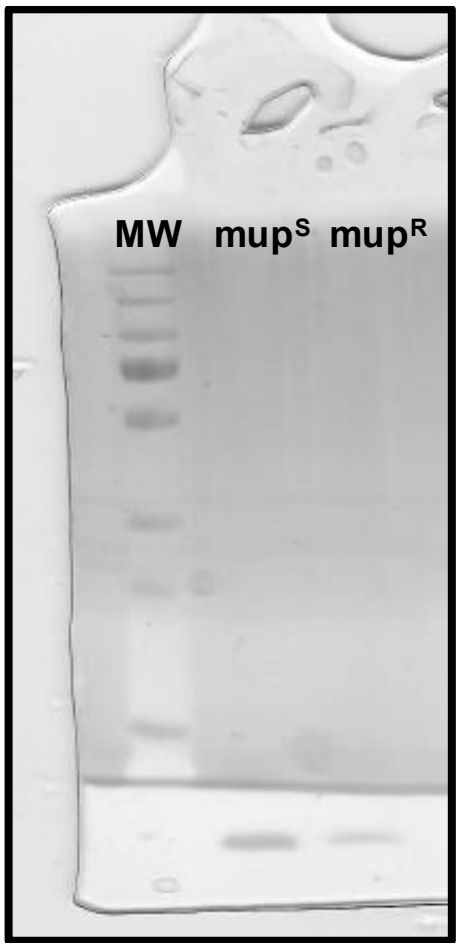

Fig. S3: Full length SDS-Page gel with PSM extractions from SH1000 (mup^S) and MY40 (mup^R). MW: molecular weight standards. Due to their small size the PSMs run in front of the loading dye front making quite a messy gel. This has been provided to explain why only a 'letterbox' snap-shot of these gels is provided in the main manuscript file.

Table S5: Strains constructed and used in this study.

Strain	**Relevant genotypic information**	**Mupirocin resistance**
SH1000	wild-type lab strain	mup^S
MY40	mupirocin resistant SH1000	mup^R
MY46	SH1000 (pPmtC)	mup^S
ROJ48	Strain 8325-4 *agr::erm*^{R}	mup^S
MY18	SH1000 *agr::erm*^{R}	mup^S
MY41	MY40 *agr::erm*^{R}	mup^R
RN6390B	wild-type lab strain	mup^S
USFL34	Clinical USA300 isolate	mup^S
MY47	Mupirocin resistant RN6390B	mup^R
MY48	Mupirocin resistant USFL34	mup^R